A Primary Lymph Node Malignancy With Features Suggestive of Dendritic Reticulum Cell Differentiation

A Report of 4 Cases

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Four cases are described of a nonlymphomatous primary lymph node malignancy characterized by the proliferation of oval and spindle cells, occasionally multinucleated, and arranged in a nesting, swirling, and storiform pattern. The combination of light-microscopic, ultrastructural, and immunohistochemical features suggests that these tumors might be derived from dendritic reticulum cells. (Am J Pathol 1986, 122:562–572)

THE MAJORITY of primary tumors of lymph nodes are of lymphocytic derivation. Of the remainder, most are composed of histiocytes-macrophages (such as malignant histiocytosis and "true" histiocytic lymphoma) or are of a vascular nature (such as nodal hemangioma and possibly Kaposi's sarcoma). It is the purpose of this communication to report 4 cases of a type of malignancy which might have arisen from yet another cellular constituent of the normal lymph node, ie, the dendritic reticulum cell.

Materials and Methods

In all 4 cases, the clinical histories were abstracted, follow-up information was obtained, and hematoxylin and eosin (H&E) slides prepared from formalin-fixed, paraffin-embedded material were reviewed. In addition, paraffin blocks were obtained from 3 cases (1, 2, and 4), and the sections prepared from them were stained with PAS, mucicarmine, reticulin, and the following immunocytochemical reactions: leukocyte common (T200) antigen (L3B12, courtesy of Dr. Ronald Levy, Stanford; PD7/26, courtesy of Dr. David Y. Mason, Oxford)[1]; dendritic reticulum cell (R4/23, courtesy of Dr. David Y. Mason)[2]; C3b complement receptor (TO5, courtesy of Dr. David Y. Mason); kappa and lambda immunoglobulin light chains (Dakopatts; 1:1600); lysozyme (Dakopatts; 1:300); α_1-antitrypsin (Cal-Biomed; 1:700); α_1-antichymotrypsin (Cal-Biomed; 1:800); Factor VIII-related antigen (Dakopatts; 1:100; *Ulex europaeus* lectin I (Dakopatts; 1:1600); actin (Miles; 1:200); desmin (Dakopatts; 1:100); vimentin (monoclonal) (Lab Systems; 1:40); neurofilaments (courtesy of Dr. D. Dahl, Boston; 1:200); neuron-specific enolase (Dakopatts; 1:300); S-100 protein (Dakopatts; 1:200); epidermal-type keratin (Dakopatts; 1:300); low-molecular-weight (44–54 kd) cytokeratin (monoclonal) (Lab Systems; 1:200); and epithelial membrane antigen (monoclonal) (Dakopatts; 1:150).

All of these stainings were carried out by using Sternberger's peroxidase-antiperoxidase (PAP) technique,[3] except for the common leukocyte and dendritic reticulum cell antigens. Binding of the monoclonal antibodies for these antigens was detected by goat anti-mouse Ig (Tago Inc., Burlingame, Calif), followed by normal swine serum and peroxidase-conjugated swine anti-goat antiserum (Tago, Inc.). An irrelevant monoclonal antibody of identical isotype was used as control.

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In addition, an electron-microscopic study was performed in the same 3 cases by examining sections stained with uranyl acetate and lead citrate that had been prepared from tissue fixed in glutaraldehyde, postfixed in osmium tetroxide, and embedded in epoxy resin.

Description of Cases

Case 1

A 40-year-old white woman from El Salvador presented in 1975 with painless right cervical lymphadenopathy, which was treated by local excision. Local recurrence developed in 1978, and this was also treated by local excision. Neither the microscopic slides nor information on the diagnosis rendered on them was available. Another local recurrence developed in 1978 (Figures 1 and 2), and a reexcision was carried out. At this time, there were no other lymphadenopathies, the peripheral blood findings were normal, and a bone marrow biopsy was negative for tumor. The cervical lesion was diagnosed microscopically as malignant lymphoma; and five courses of chemotherapy consisting of bleomycin, doxorubicin, cyclophosphamide, vincristine, and prednisone were administered. Several additional recurrences in the right cervical region developed in 1980 and 1981, and these were treated by further local excisions. In 1982, ie, 7 years after the initial presentation, a 10-cm tumor mass was found in the liver. Chemotherapy was reinstituted (type and dosage unknown), but the patient died shortly thereafter. No autopsy was performed.

Case 2

A 29-year-old man from Argentina presented in 1984 with a 4-month history of painless left cervical adenopathy, which was treated by local excision. Additional biopsies were obtained from slightly enlarged cervical and inguinal lymph nodes, but these showed no pathologic changes. Bone marrow and peripheral blood studies revealed no abnormalities. No further therapy was instituted because of the uncertainty of the diagnosis. The patient is alive and well, with no evidence of recurrent adenopathy, 1 year after the initial biopsy.

Case 3

A 58-year-old white woman from Belgium presented in 1973 with left cervical adenopathy, which was excised and diagnosed microscopically as "lymphosarcoma." This was followed by the appearance of other enlarged nodes in the neck and axillae, which were not biopsied. Treatment with four cycles of doxorubicin, vincristine, and prednisone was instituted. The patient was subsequently lost to follow-up.

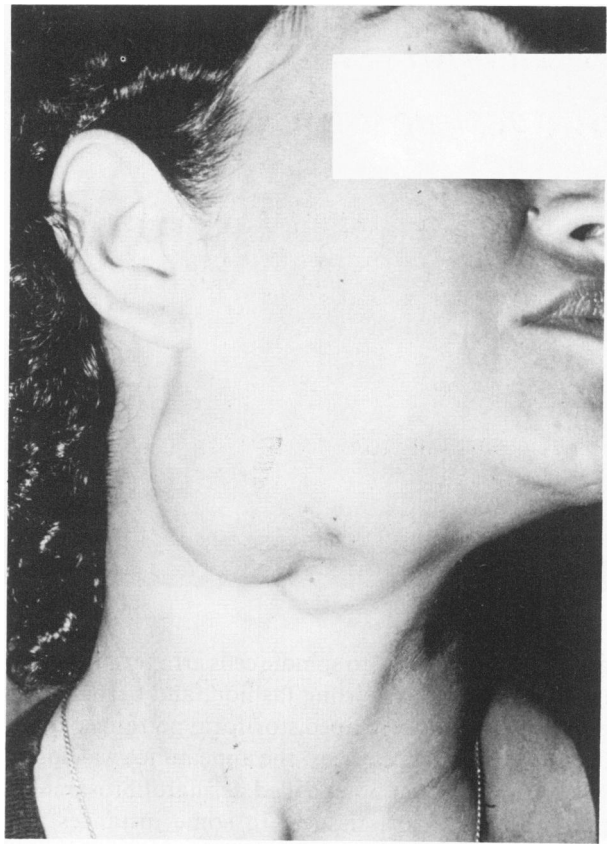

Figure 1—Upper cervical adenopathy, representing the second recurrence of the tumor. The surgical scar from the previous excision is evident (Case 1).

Case 4

A 38-year-old white man from Mexico presented in 1979 with left cervical adenopathy, which was treated by excision. This was diagnosed initially as malignant lymphoma and at another institution as metastatic hemangiopericytoma. A thorough workup including exploratory laparotomy was negative. No further therapy was instituted. Local recurrence developed in 1984; this was interpreted microscopically as metastatic malignant fibrous histiocytoma, and a wide local excision was carried out. No other adenopathies or hepatomegaly were noted. Peripheral blood and bone marrow were unremarkable. There is no evidence of disease 4 months after the excision of the recurrence. Radiation therapy to the left side of the neck is being planned.

Pathologic Findings

Light Microscopy

The microscopic features of these 4 cases were very similar and are therefore described together. There was a partial to complete effacement of the architecture by

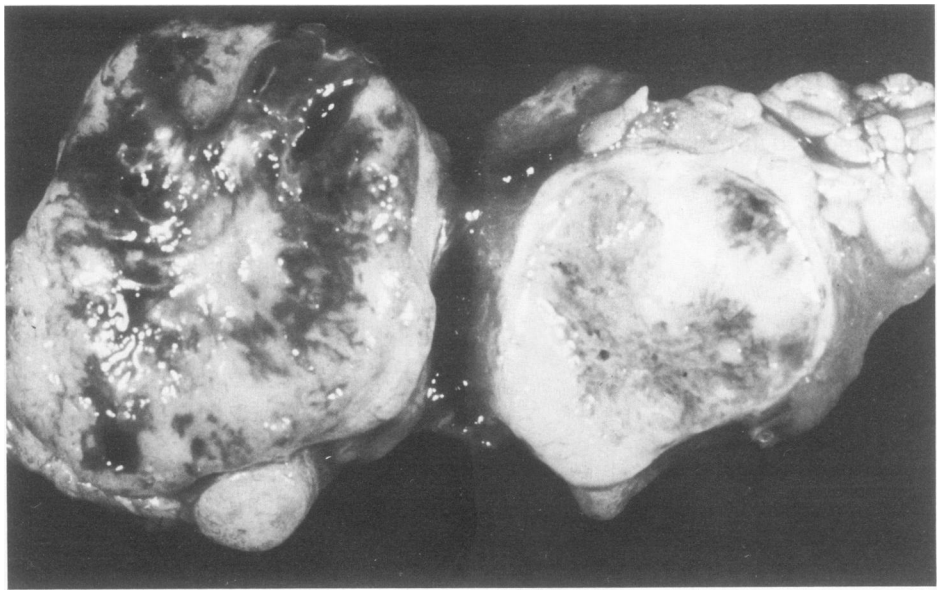

Figure 2—Cut surface of the involved node. The appearance is variegated, with a predominance of white solid areas of firm consistency, alternating with foci of hemorrhae (Case 1).

a proliferation of oval to spindle cells arranged in a nesting, whorling, and swirling fashion, and occasionally exhibiting a well-developed storiform pattern (Figures 3 and 4). In the latter areas, the appearance was somewhat similar to that of so-called dermatofibrosarcoma protuberans of soft tissues. In some instances, the proliferation replaced the entire nodal parenchyma; in others, the involvement was focal, with sharp boundaries from the residual lymphoid tissue, which appeared in the form of follicles or perivascular collections of lymphocytes (Figure 5). A sprinkling of mature lymphocytes was constantly found among the tumor cells,

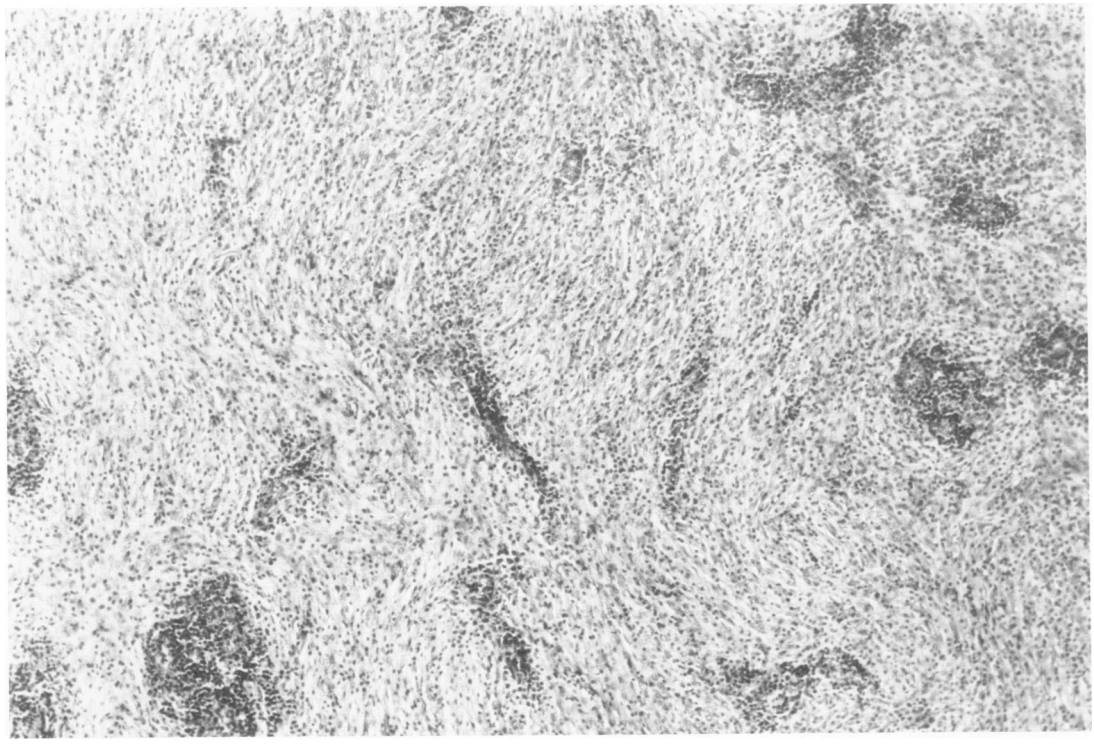

Figure 3—The lymph node structure is largely replaced by a monomorphic population of pale oval cells. The residual lymphoid tissue is limited to small follicles and perivascular collections of lymphocytes (Case 1).

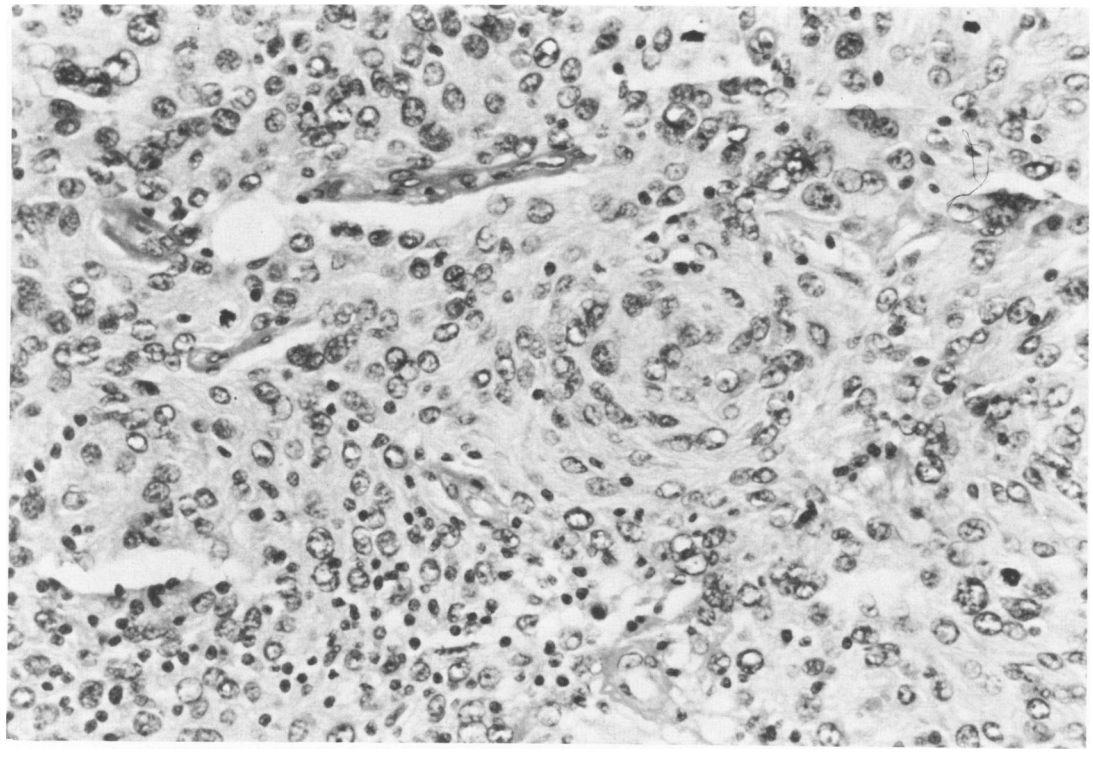

Figure 4—The tumor cells are arranged in ill-defined whorls. The nuclear shape varies from round to oval. A sprinkling of mature lymphocytes is seen among the tumor cells (Case 1).

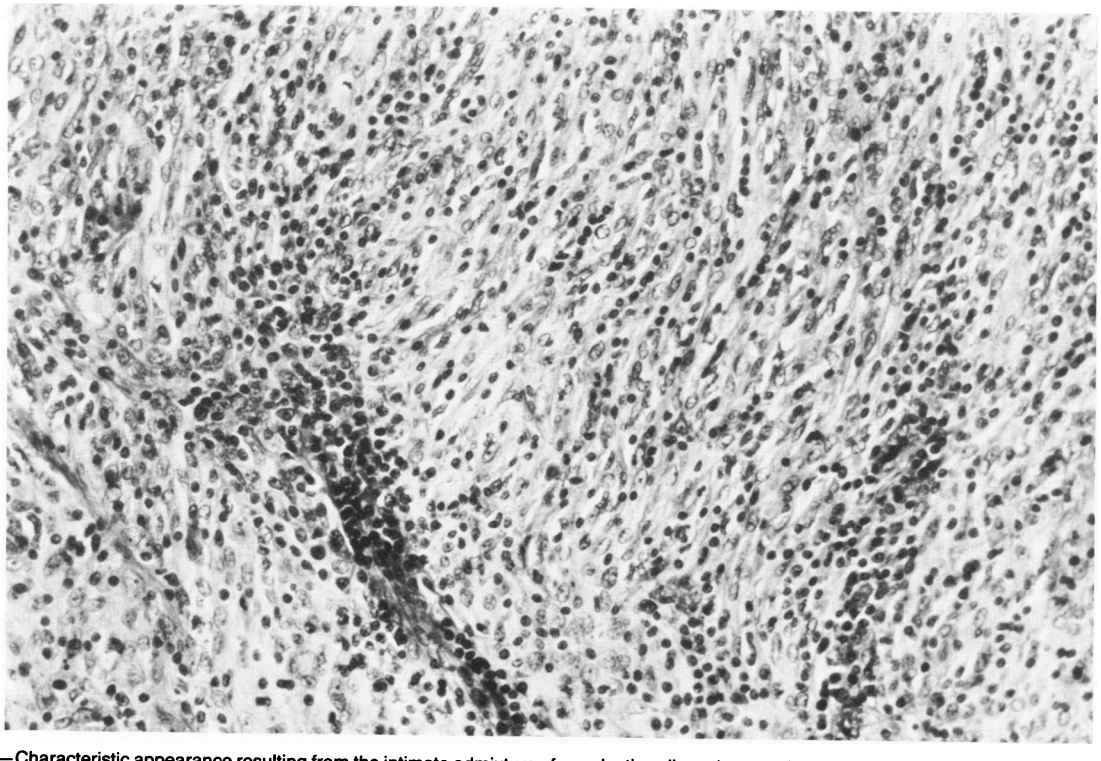

Figure 5—Characteristic appearance resulting from the intimate admixture of neoplastic cells and mature lymphocytes. The latter are concentrated around blood vessels (Case 1).

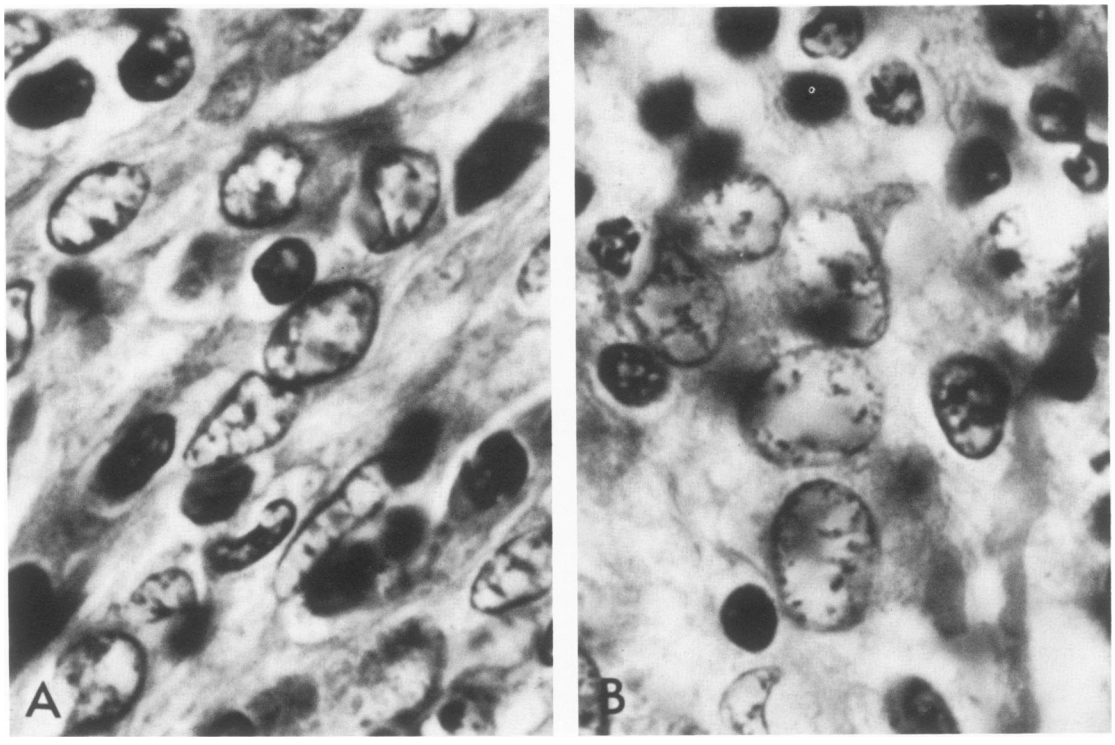

Figure 6—High-power view of the tumor cells. The nuclei are large and vesicular, with scanty chromatin and inconspicuous nucleoli. Their shape varies from spindle (**A**) to round (**B**), with oval forms predominating. The cytoplasmic borders are indistinct (Case 1).

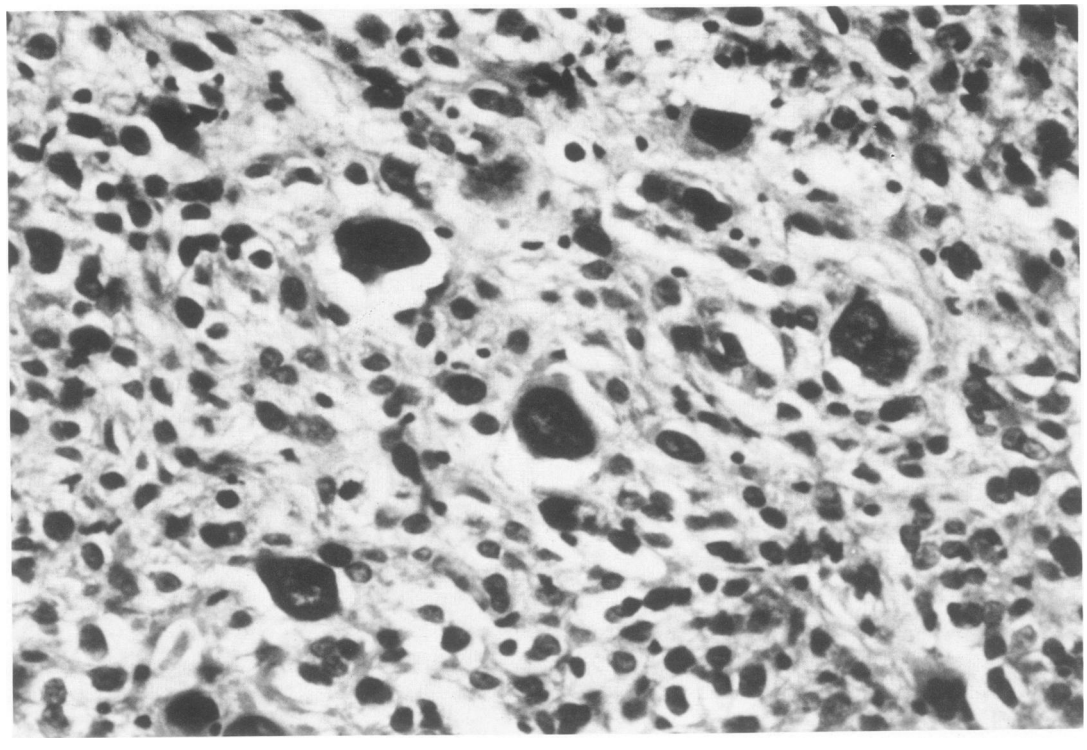

Figure 7—A more anaplastic appearance of the tumor is obvious in the last lymph node recurrence, with the presence of highly atypical nuclear forms (Case 1).

the resulting picture being reminiscent of that seen in thymoma (Figure 6). The tumor cells were characterized by oval or spindle vesicular nuclei with smooth contours and inconspicuous nucleoli (Figure 6). Mitotic activity was scanty to moderate. The cells had a moderate amount of pale eosinophilic, slightly fibrillary cytoplasm; the cell margins were indistinct. Blood vessels of capillary size were often seen in the midst of the neoplastic elements. A variable number of tumor cells were multinucleated, their appearance being very similar to that of the so-called polykaryocytes or Warthin–Finkeldey giant cells, as described in a variety of reactive and neoplastic lymph node disorders.[4]

Necrosis was consistently absent. The peripheral sinuses were obliterated, and the neoplastic proliferation sometimes extended outside the boundaries of the node.

Sequential lymph node biopsies available in Case 1 showed microscopic progression of the disease, manifested by increased cellularity, nuclear pleomorphism and hyperchromasia, and the presence of numerous mitotic figures, some of them atypical (Figure 7). The tumor was particularly pleomorphic and sarcomalike in the last recurrence in the neck and in the nodule taken at biopsy from the liver during the terminal phase of the disease.

Special Stains

The tumor cells were negative for periodic acid-Schiff (PAS) and mucicarmine. Reticulin stains showed that groups of tumor cells were surrounded by reticulin fibers.

Of all the immunohistochemical reactions performed, tumor cell reactivity was only identified for the leukocyte common (T200) antigens, the dendritic reticulum cell antigen (R4/23) and the C3b complement receptor (TO5) (Table 1). All 3 cases tested expressed leukocyte common (T200) antigens, providing evidence for hematolymphoid origin (Figure 8A and B). Two of the 3 cases tested also expressed dendritic-reticulum-cell-related antigens (Figure 8C and D). In our experience, normal dendritic reticulum cells have shown consistent, although variable, positivity for these markers in lymph node preparations obtained from paraffin-embedded tissue, particularly in Bouin-fixed material (Figure 8E and F).

Electron Microscopy

The nuclei of the tumor cells were plump, ovoid, and smoothly contoured, with peripherally marginated chromatin and a single small to medium-sized nucleolus (Figure 9). Cytoplasmic organelles included scattered ribosomes and polyribosomes, poorly developed rough endoplasmic reticulum, numerous short profiles of smooth endoplasmic reticulum, scattered mitochondria, and an inconspicuous Golgi complex. Lysosomes were very scanty. Basal lamina, pinocytotic vesicles, dense core secretory granules, tonofilaments, and Weibel–Palade bodies were uniformly absent. The most striking feature of the tumor cells was the presence of long, complex, undulating cytoplasmic extensions (Figure 10). These intertwined and connected with each other through numerous cell junctions, most of which were of the macula adherens type (Figure 11). This combination of features was present in varying degrees in all 3 cases examined.

Table 1—Results of Immunohistochemical Reactions in Sections From Formalin-Fixed, Paraffin-Embedded Material

Case	Leukocyte common (T200) (L3B12)	Leukocyte common (T200) (PD7/26)	Dendritic reticulum cell (R4/23)	C3b complement receptor (T05)
1 (1978)	2+	1+	2+	1+
1 (1981)	2+	1+	1+	±
2	1+	–	–	–
3	ND	ND	ND	ND
4	2+	1+	2+	1+

–, negative staining; ±, weak to equivocal staining, compared with isotype-matched control; 1+, 10–50% of cells stained with at least moderate intensity; 2+, over 50% of cells stained with at least moderate intensity—this represents the most positive result in this scale; ND, not done.

Discussion

The clinical presentation of these 4 cases was very similar, in the sense that all patients had initially painless unilateral cervical adenopathy as the only abnormal physical finding. They were all asymptomatic, no other lymphadenopathy or organomegaly was noted at presentation, and laboratory tests were unremarkable.

Microscopically, the differential diagnosis of the neoplastic disorder represented by these cases includes the following entities: metastatic carcinoma (particularly from the nasopharyngeal tumor known as "lymphoepithelioma"), ectopic thymoma, metastatic malignant melanoma, primary and metastatic malignant fibrous histiocytoma, malignant schwannoma, Kaposi's sarcoma, and malignant lymphoma. We believe that the combination of light-microscopic appearance, electron-microscopic features, and the immunohistochemical profile (positivity for leukocyte common antigens and dendritic reticulum cells and lack of reactivity for all other markers) effectively rules out all of these possibilities. Specifically, we have found that malignant fibrous histiocytoma of the soft tissues is negative for T200 and C3b receptor antigens.

Because the evolution of these cases strongly suggests that they represent primary lymph node malignancies,

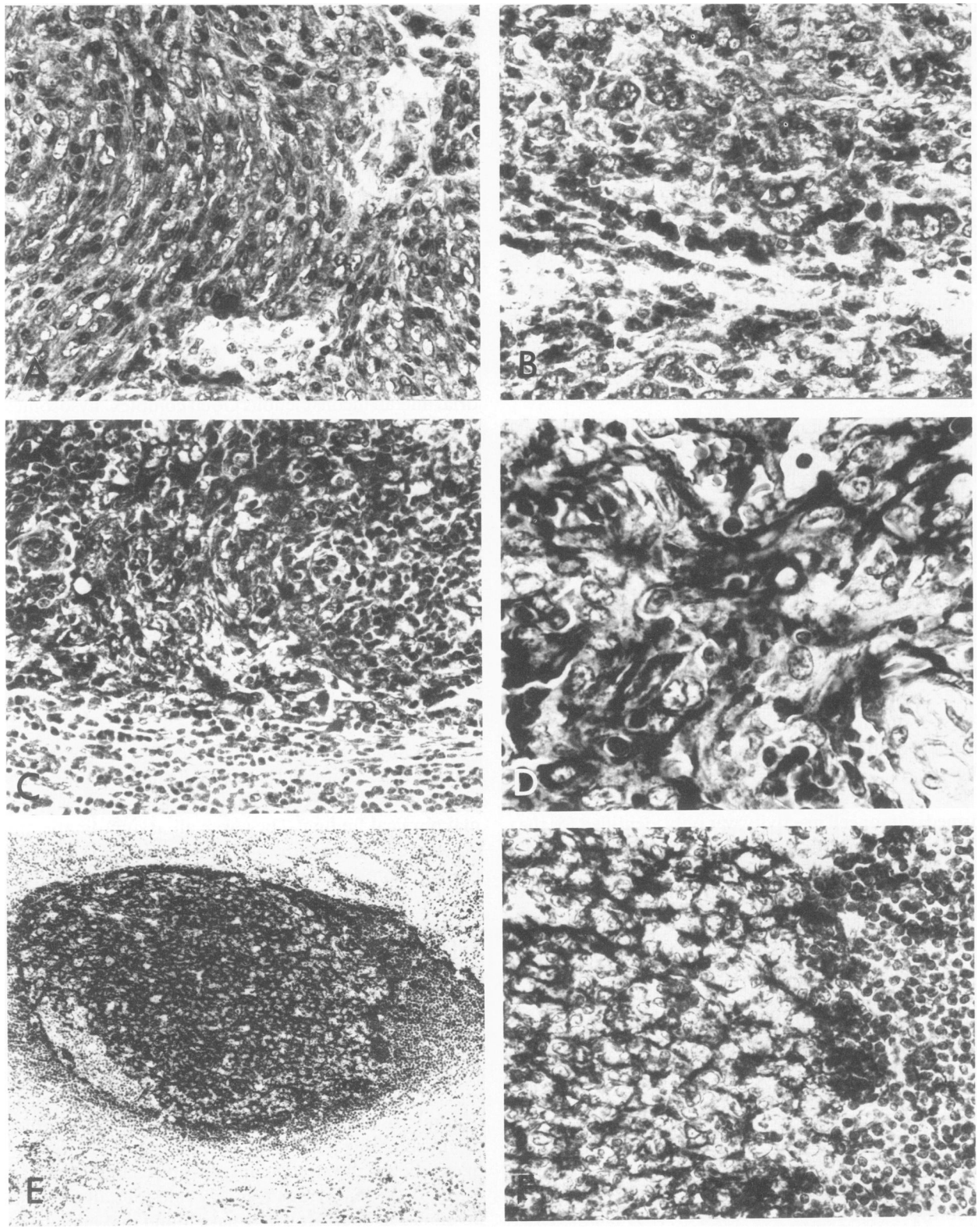

Figure 8A—The tumor cells show positivity for leukocyte common antigen. (L3B12, Case 1, ×400). **B**—The tumor cells in the recurrent tumor from the same case have a more pleomorhic appearance, but they retain positivity for leukocyte common antigen. (L3B12, Case 1, ×400). **C**—The tumor cells stain darkly for the dendritic reticulum cell antigen. Note the adjacent unstained lymphocytes. (R4/23, Case 4, ×320). **D**—Strong staining for C3b receptor of the cell processes of the tumor cells. (T05, Case 4, ×640). **E**—The normal dendritic reticulum cells in a germinal center from a Bouin-fixed tonsil stain intensely for C3b receptor. (T05, ×200) A similar degree of positivity, although of a more diffuse nature, was observed in the same cells for the dendritic reticulum cell antigen (R4/23). **F**—Higher magnification of the same preparation, highlighting the dendritic configuration of the C3b receptor-positive cells. (T05, ×400)

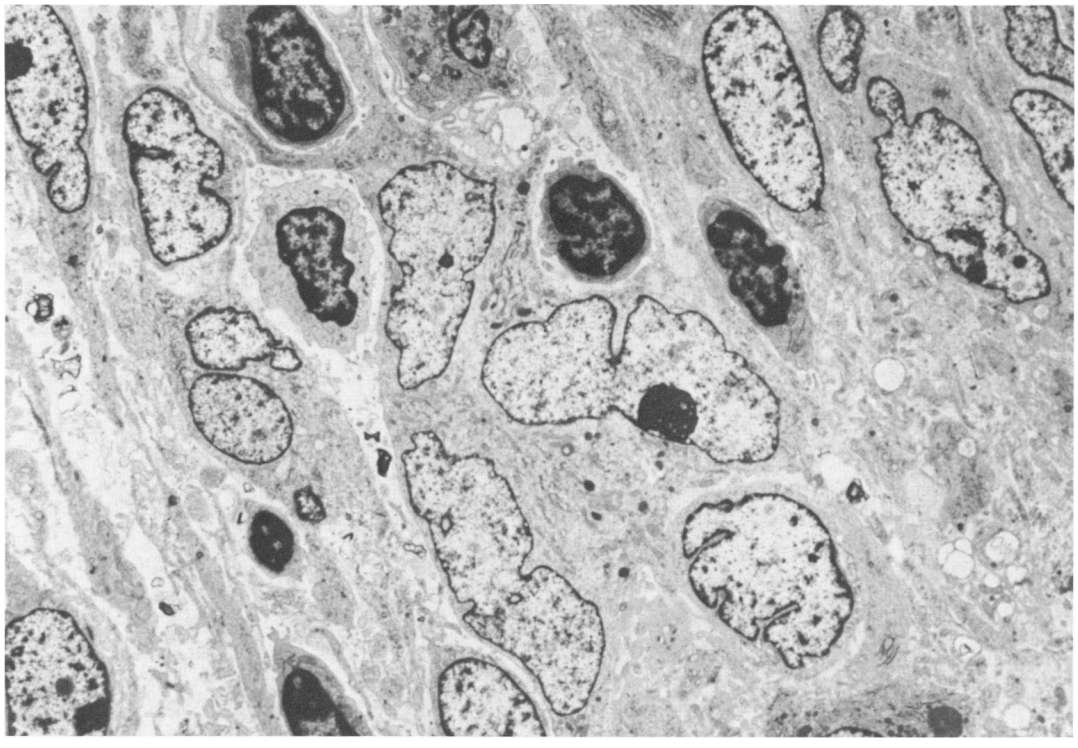

Figure 9—Low-power electron micrograph of tumor cells, showing nuclear indentations, scanty and finely dispersed chromatin, occasionally prominent nucleoli, and a moderate amount of cytoplasm. Several small lymphocytes are seen between the tumor cells (Case 1). (×3100)

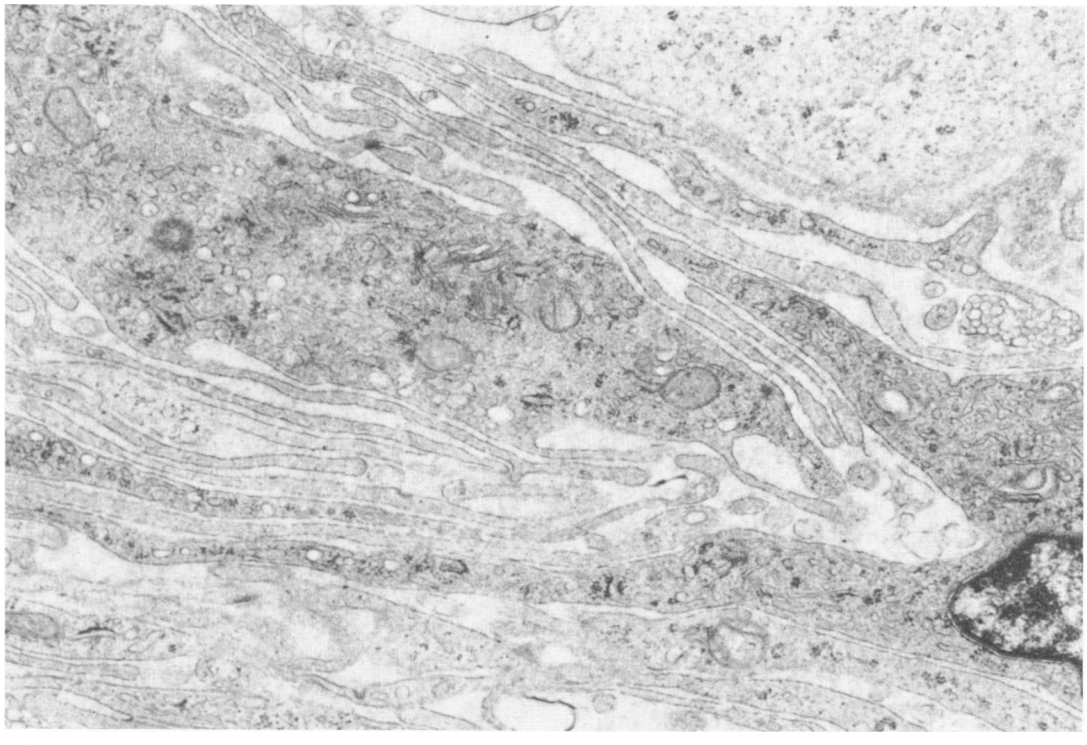

Figure 10—Extremely elongated cell processes from several tumor cells intertwine with each other (Case 1). (×16,850)

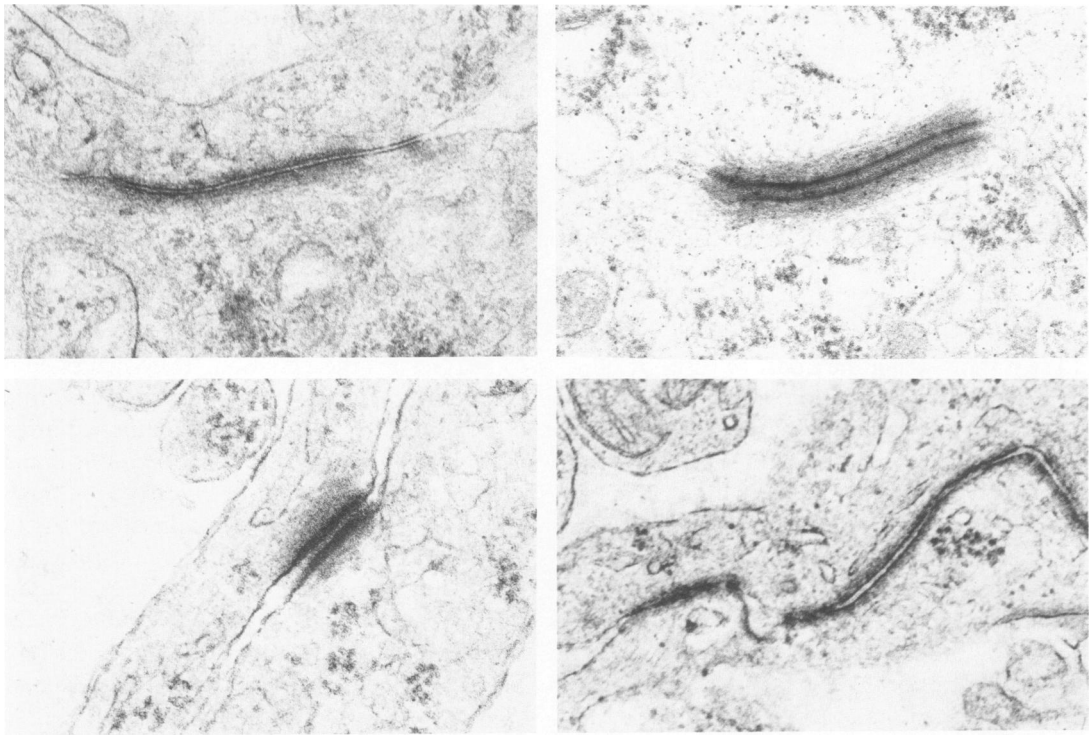

Figure 11—Specialized cell junctions present between tumor cells. They vary in length and complexity, but most have the features of maculae adherentes (desmosomes) (Case 1). (×40,150)

we compared the light-microscopic, ultrastructural, and immunohistochemical features of the tumor cells with those of all the known normal cellular constituents of the lymph node and found that these features matched very closely those of one particular variety of reticulum cell.

Four types of reticulum cells have been described in normal lymph nodes and designated respectively as dendritic (DRC), interdigitating (IRC), histiocytic (HRC), and fibroblastic (FRC).[5-8] These cells have been distinguished from each other by a combination of morphologic, immunohistochemical, enzyme-histochemical, and ultrastructural features. DRCs, first described by Maximow in 1927 as "embryonal nonphagocytic reticulum cells,"[9] are located in the B areas of the normal lymph nodes. Their greatest concentration is in the lighter zone of the germinal center, but they are also present in the darker zone and in the surrounding mantle zone.[6] Ultrastructurally, they have long, branching cell processes with well-formed desmosomes forming a meshwork in which B lymphocytes nest.[10,11] Their convoluted cell membranes contain Fc and complement receptors, which enable DRCs to trap antigen–antibody complexes passing through the germinal center.[12,13] It has been speculated that DRCs present antigens to B lymphocytes during the development of the immune response. Recently, a monoclonal antibody (R4/23) has been developed which is claimed to be almost specific for DRCs and which appears to react with the complement receptors located on the cell membrane.[2] Another monoclonal antibody (Ki-M4) is said also to specifically recognize DRCs and their possible precursors in blood.[14]

IRCs are permanent residents of the T-cell areas (paracortex) of nodes. They resemble Langerhans cells in their enzymatic features and immunohistochemical positivity for S-100 protein, but lack Birbeck's granules.[15,16] Their function is not fully understood; they are presumed to stimulate (and perhaps present antigen to) T lymphocytes.

HRCs, traditionally referred to as "tingible-body macrophages" when located in the germinal center and exhibiting phagocytosis of nuclear debris, are the only actively phagocytic cells of the group.[5] They are readily distinguished from the others because of their high content of hydrolytic enzymes. The usual immunohistochemical markers used for their identification are lysozyme, α_1-antitrypsin, and α_1-antichymotrypsin.

FRCs are cells scattered throughout the node which are closely related (and perhaps identical) to fibroblasts and myofibroblasts. They contain abundant alkaline phosphatase, produce collagen, and have a basically structural function.[5]

Of these four cell types, only the DRCs matched the

features we encountered in the cells of our tumors, particularly with regard to the ultrastructural properties and the immunohistochemical profile. Specifically, DRCs are the only elements of the node which are joined by specialized junctions of desmosomal type and which are known to react with the monoclonal antibody R4/23. Also in keeping with this possibility is the presence in all of our cases of multinucleated tumor cells, in view of the fact that this has been shown to be a property of normal DRCs.[17] The only feature not fully consonant with this interpretation was the distribution of the tumor within the node. In cases of partial involvement, the concentration of tumor cells was not in the areas corresponding to germinal centers (as one would expect from a tumor of B zone-dependent cells) but rather exhibited a diffuse or an interfollicular pattern. We therefore considered the alternative possibility of an origin from yet another type of normal lymph node element, ie, the sinus lining cell or littoral cell. However, this cell does not possess desmosomal junctions and is thought to be nonreactive with the monoclonal antibody R4/23.

Reported cases of tumors of reticulum cells are very few, probably because of their extreme rarity, but perhaps also because cases of this entity may have been variously interpreted as metastatic carcinoma, ectopic thymoma, and primary or metastatic malignant fibrous histiocytoma.

Van der Valk et al[18] described 4 cases of a lymphoreticular malignancy which they interpreted as of DRC derivation. The sites involved were lymph nodes (submandibular, supraclavicular, and axillary), tonsil, bone marrow, spleen, kidney, omentum, and ovary. Two of the patients had Stage IV disease at presentation. By light microscopy, these cases were very different from ours, in that the overall appearance was essentially that of a large cell lymphoma. The tumor cells were generally positive for Ia-like antigen, complement receptors (C3), 5-nucleotidase, adenosine triphosphatase (ATPase), and α-naphtyl acetate esterase, and generally negative for Fc, lysozyme, α_1-antitrypsin, α_1-antichymotrypsin, acid phosphatase, and surface and cytoplasmic immunoglobulins. Ultrastructurally, the cells had interweaving slender villous processes and primitive cell junctions of the zonula adheraens type, but desmosomes were not found. Although some of the immunologic and ultrastructural features were suggestive of DRCs, the lack of desmosomal junctions and the positivity for ATPase cast some doubts on the author's proposal.

A neoplasm interpreted as of IRC type was reported by Feltkamp et al.[19] The tumor cells were pleomorphic, with spindle and round cell forms. The nuclei were bean-shaped or strongly lobulated, instead of having the smooth outlines seen in our cases. There was positivity for Ia-like antigens and high levels of ATPase activity. Ultrastructurally, the cells had bladelike indentations and were surrounded by broad cellular protrusions. Desmosomal connections were not described. The Golgi apparatus was very elaborate, whereas in our cases it was poorly developed. The nuclei were often bizarre, with deep invaginations.

Additional examples of tumors of probable reticulum cell derivation were discovered by Turner et al[20] in a review of 14 malignancies with morphologic features of histiocytic differentiation. They suggested that 2 of these tumors (showing ATPase and S-100 protein reactivity) were consistent with IRC differentiation, and 1 (showing positivity for alkaline phosphatase) was related to FRC. They found no cases with features suggestive of DRC differentiation. In connection with this article, it is pertinent to point out that in our Case 1 the microscopic appearance of the tumor during the terminal stages of the disease was quite similar to that of a pleomorphic "histiocytic" lymphoma, even if the initial specimen did not resemble at all this tumor type.

It is our impression that none of the cases described by the above authors match the features seen in our patients. It is also our belief that the neoplastic disorder exemplified by these 4 cases represents a distinct and recognizable pathologic entity. We postulate a possible origin from DRCs on the basis of morphologic, ultrastructural, and immunohistochemical similarities between the tumor cells and this member of the "reticulum cell" family.

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